CHAPTER 1

GENERAL INTRODUCTION

1.1 Maize breeding in Ethiopia: Historical overview

Maize was first introduced to Ethiopia by the Portuguese in the 16th or 17th century (Hafnagel, 1961). Since its introduction, it has gained importance as a food and feed crop in Ethiopia. Averages of the 2000/2001 national production estimates of the Central Statistical Authority (CSA, 2001) indicate that maize, with 1.40 million hectares and 2.52 million tons, accounts for about 20.9% of the total area and 32.6% of the gross annual grain production. Maize is one of the cereals that provide calorie requirements in the traditional Ethiopian diet. It is prepared and used as unleavened bread, roasted and boiled green ears, parched mature grain porridge and in local drinks like 'tella', 'borde' and 'areke' (Mulatu *et al*., 1992). Apart from these uses, maize leaves are fed to animals, while dry stalks are used as fuel and for the construction of fences and huts.

Maize growing areas in Ethiopia are broadly classified into four ecological zones based on altitude and annual rainfall (EARO, 2000). These are (1) the high altitude moist zone, which receive 1200 to 2000 mm rainfall and is at an altitude of 1700 to 2400 meters above sea level (masl), (2) the mid-altitude moist zone (1200-2000 mm rainfall and an altitude of 1000 to 1700 masl), (3) the low-altitude moist zone (less than 1000 masl and 1200-1500 mm rainfall), and (4) the moisture stress zone (from 500 to 1800 masl and receive less than 800 mm rainfall).

Maize breeding in Ethiopia has been ongoing since the 1950's and has passed through three distinctive stages of research and development (Mulatu *et al*., 1992). These are (1) from 1952 to 1980, the main activities were the introduction and evaluation of maize materials from different part of the world for adaptation to local conditions, (2) from 1980 to 1990, the work was focused on evaluation of inbred lines and development of hybrid and open-pollinated varieties, and (3) from 1990 to present, the main activities were (a) extensive inbreeding and hybridization, (b) development of early maturing or drought tolerance cultivars, and (c) collection and improvement of maize with adaptation to highland agroecologies. As a result, various improved hybrids and open-pollinated varieties were released for large-scale production, especially for mid-altitude zones. The highland maize breeding program was started in 1998 in collaboration with the International Maize and Wheat Improvement Center (CIMMYT). The initial objectives of the program were facilitating collection, evaluation and documentation of locally important highland maize accessions in eastern African countries.

1.2 Importance of maize in the highlands of Ethiopia

The highland areas of Ethiopia constitute 36% of the total land area, over 90% of the crop land and support about 88 and 70% of the human and the livestock population, respectively (CSA, 1998). In these regions, 20% of the total land was devoted to maize cultivation and more than 30% of small-scale farmers depend on maize for their livelihood (CSA, 1998). In light of the rapidly increasing human population and expansion of agriculture into the highland areas of Ethiopia, maize has been selected as one of the national commodity crops (due to its high yield potential and wide adaptation) for the food self-sufficiency program of the country (Mulatu *et al.*, 1992).

Maize cultivars that are used in the highland regions of Ethiopia are well adapted, but low yielding, open-pollinated varieties developed by local farmers. Many of these varieties resulted from centuries of planting, harvesting and selection. The highland maize varieties may be grouped into a number of completely or partially isolated populations, which may each be adapted to different highland conditions. Unfortunately, formal breeding programs have had little success in developing improved varieties for these diverse agroecologies. In the past decade, only two improved open-pollinated maize varieties were developed for the highland zone (Twumasi-Afriyie *et al*., 2001).

In view of the above, the Highland Maize Germplasm Collection Mission was launched throughout the highlands of Ethiopia in collaboration with CIMMYT in 1998 (Twumasi-Afriyie *et al*., 2001). As part of this project, 287 maize accessions were collected from farmers' fields throughout the highland regions of Ethiopia. Currently, there is no information on the extent of morphological and genetic variation among these accessions.

1.3 Objectives and outline of the study

Molecular markers are rapidly being adopted by crop improvement researchers globally as effective and appropriate tools for basic and applied studies in crop plants. Use of molecular markers in maize breeding ranged from facilitating appropriate choice of parents for crosses, to mapping/tagging of gene blocks associated with economically important traits often termed as quantitative trait loci (QTLs).

In this PhD study, Amplified Fragment Length Polymorphism (AFLPs), microsatellites or Simple Sequence Repeats (SSRs) and agro-morphological traits were employed for genetic analysis of traditional Ethiopian highland maize accessions. The general objectives of this study were (1) to classify the highland maize accessions into distinct groups based on genetic profiles and morphological traits, (2) to test the utility of bulking DNA (by bulking leaf samples) for large-scale genetic characterization of open-pollinated varieties, (3) to determine the correlation between estimates of genetic diversity measured by AFLPs, SSRs and morphological traits, and (4) to study population level association of molecular markers with quantitative trait variation in diverse maize accessions. The specific objectives and research methodologies for each experiment is presented in detail under each chapter.

In chapter 2, the importance of maize for genetic studies, methods and measures for assessing genetic variation, and application of molecular markers in crop improvement are reviewed. In chapter 3, the phenotypic diversity of traditional Ethiopian highland maize accessions is presented. Multivariate statistics were employed to classify the accessions into similar phenotypic groups. In addition, different statistical measures such as phenotypic and genotypic variability, broad sense heritability and genetic advance were calculated. This chapter allowed the selection of representative maize accessions from all agroecologies and phenotypic classes that were used for subsequent molecular analyses.

In chapter 4, bulked-AFLPs were employed to study genetic diversity and relationships of selected maize accessions. The results of this chapter highlight the use of bulked leaf samples for large-scale genetic characterization of open-pollinated varieties, which are commonly cultivated in developing countries. These investigations are continued in chapter 5. In this chapter, the utility of analyzing microsatellites on agarose gels and bulking leaf samples for large-scale diversity study is investigated. The results indicated that bulking leaves from 15 randomly selected individuals per accession proved to be useful for the creation of representative templates for DNA extraction, thereby reducing the cost of DNA extraction and subsequent genotyping.

In chapter 3, 4 and 5, detailed phenotypic and marker information were generated for a selected set of traditional Ethiopian highland maize accessions. The question is how can this information be used in an efficient way for phenotypic and genetic classification of the materials? The answer was highlighted in chapter 6, which deals with the agreement between distance estimates based on molecular markers and morphological traits. The results showed that significant correlations between different data set and validate the use of these data to calculate the different diversity statistics for Ethiopian highland maize accessions. In chapter 7, the association of SSRs with quantitative trait variation was investigated in the selected set of highland maize accessions. The information obtained from this chapter will be useful for future association genetic studies in similar populations. Finally, chapter 8 presents a general discussion of the implication of the results of this study for breeding, future collections and conservation of maize in the highlands of Ethiopia.